Published online October 27, 2005

Molecular Marker Satt481 is Associated with Iron-Deficiency Chlorosis Resistance in a Soybean Breeding Population

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ABSTRACT

Soybean [*Glycine max.* (L.) Merr.] breeders have improved resistance to iron-deficiency chlorosis (IDC) using conventional breeding approaches; however, many IDC-resistant cultivars have lower yields compared to IDC-susceptible cultivars. The importance of environment on IDC-resistance expression hinders progress in breeding for resistance. An environment-independent selection strategy, such as marker-assisted selection (MAS), may increase breeding efficiency. Our objective was to determine whether simple sequence repeat (SSR) markers located in previously reported quantitative trait loci (QTL) for IDC resistance would be associated with IDC resistance in a breeding population. One-hundred and eight SSR markers genetically linked to eight QTLs on eight molecular linkage groups (MLGs) previously identified for IDC were tested in a breeding population evaluated for IDC resistance on calcareous soils in Iowa. The breeding population was developed from a cross between Pioneer 9254 and A97–770012. The F_2 lines were genotyped with markers and the F_2–derived lines ($F_{2:4}$ and $F_{2:5}$) were evaluated for IDC resistance. Three markers were associated with IDC resistance: Satt211, Satt481, and Sat_104. However, of the three markers, only Satt481 was associated to IDC resistance across environments. Although Satt481 accounted for only 12% of the total phenotypic variation, molecular analysis of the eleven-most resistant lines in the population indicated that 73% of the lines were homozygous for the resistant allele at the Satt481 locus. Our results indicated that Satt481 may be useful to improve IDC resistance in this soybean population and that additional QTLs conferring resistance to IDC might exist in soybean.

IRON-DEFICIENCY CHLOROSIS of soybean may occur when certain cultivars are grown on calcareous soils (Froehlich and Fehr, 1981; Niebur and Fehr, 1981). The cultivars, unable to acquire and utilize iron efficiently, may develop foliar chlorosis, leading to yield loss (Froehlich and Fehr, 1981; Niebur and Fehr, 1981). Calcareous soils in the USA are found mainly in the Midwestern portion of the country, especially Iowa, Minnesota, Nebraska, and South and North Dakota (Franzen and Richardson, 2000; Froehlich and Fehr, 1981; Inskeep and Bloom, 1984; Penas and Wiese, 1990).

The best method of preventing IDC is to plant IDC-resistant cultivars (Fehr, 1982). Although resistance to IDC in soybean has been improved through conventional breeding approaches (Cianzio, 1991; Cianzio and Voss, 1994; Hintz et al., 1987), IDC-resistant lines may have lower yield potential than IDC-susceptible cultivars (Fehr, 1982, 1983).

Selection for IDC resistance has been based on foliar-chlorosis symptoms observed in plantings on calcareous soils (Cianzio et al., 1979). Complex polygenic inheritance of IDC resistance and genotype × environment interactions may result in inaccurate assessment of resistance, which reduces the value of phenotypic rating and decreases efficiency of breeding to improve the trait (Lin et al., 2000b). Therefore, if an environment-independent approach could be devised, there might be the potential to improve breeding efficiency for IDC.

Quantitative trait loci for IDC resistance have been identified using restriction fragment length polymorphism (RFLP) markers in two soybean populations in field and hydroponic evaluations (Lin et al., 1997, 2000a). Lack of common RFLP markers in the two populations prompted Lin et al. (2000b) to conclude that the examined molecular markers would be inefficient in MAS. Instead, they proposed use of SSR markers due to high occurrence of polymorphisms and the availability of large number of SSR markers in the public domain.

The mapping populations used by Lin et al. (1997, 2000a) were developed by using highly IDC-susceptible genotypes crossed to highly IDC-resistant genotypes. However, there is no information about the associations of QTLs and IDC resistance in actual breeding populations, namely when the parents used in the cross possess a number of desirable agronomic traits along with moderate IDC resistance. To simulate a practical breeding program, a population was developed as described above, using parents with intermediate IDC scores and a number of desirable agronomic traits, to examine the association between QTLs and IDC using SSR markers. Preliminary results from this population, using 1-yr data, indicated a possible association between Satt481 and IDC resistance (Charlson et al., 2003). On this basis, our objective was to continue examining whether SSR markers located in previously reported IDC QTLs would be associated with IDC resistance in the breeding population.

MATERIALS AND METHODS

Population Development

A breeding population was developed from a cross between a high-yielding cultivar, Pioneer 9254 (P9254), and an advanced

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Published in Crop Sci. 45:2394–2399 (2005).
Crop Breeding, Genetics & Cytology
doi:10.2135/cropsci2004.0510

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Abbreviations: H_b^2, Broad-sense heritability; IDC, iron-deficiency chlorosis; MAS, marker-assisted selection; MLG, molecular linkage group; QTL, quantitative trait loci; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat.

experimental line, A97–770012, developed for IDC resistance. Pioneer 9254 was identified from the 1997 Iowa Crop Performance Test. A97–770012 is an experimental line from Iowa State University derived from a breeding population developed from the cross of Northrup King 'S20–20' by Asgrow 'A2234'.

The cross, population development, and seed increases were conducted at Isabela, PR, on noncalcareous soils. Several F_1 seeds were harvested during winter 1997, which were planted during the 1998–1999 season in Puerto Rico. Seven F_1 plants were grown, and the F_2 seed of each F_1 plant was harvested individually. While maintaining the identity of the F_2 lines, 145 F_2 plants resulting from the seven F_1 plants were self-fertilized for two successive generations to produce 145 $F_{2:4}$ lines for 2000 and 2001 field evaluations. The $F_{2:4}$ lines were advanced to the $F_{2:5}$ generation for 2002 field evaluations.

Phenotypic Evaluation of IDC Resistance

The parents, F_2–derived lines ($F_{2:4}$ and $F_{2:5}$ lines), and five IDC check cultivars (BSR101, Century 84, Corsoy 79, Kenwood 94, and Williams 82) were evaluated for IDC resistance on calcareous soils at two Iowa locations, Ames and Humboldt, during the summers of 2000, 2001, and 2002. The soil at each location is described as Harps loam (fine-loamy, mixed, superactive, mesic Typic Calciaquolls) and soil pH averages 8.0. In 2000, plots were planted at Ames on 22 May and Humboldt on 23 May. In 2001, plots were planted at Ames 8 June and Humboldt on 18 June. In 2002, plots were planted at Ames 29 May and Humboldt 30 May. Each genotype was randomly assigned to a plot. Plots were 75-cm-long, single rows planted with 25 seeds at a rate of one seed per 3 cm. At each location, plots were organized in a randomized complete block design with three replications.

Evaluation of IDC resistance was conducted at the V4 stage of growth (Fehr and Caviness, 1981) by visually rating foliar chlorosis using a scale of 1.0 to 5.0 with 0.5 increments, where 1.0 represented no yellowing of the leaves and 5.0 indicated severe yellowing with necrosis and plant death (Cianzio et al., 1979). Each plot was given a chlorosis score on the basis of the average amount of chlorosis observed for all plants in the plot.

Analyses of variance were conducted with all main and interaction effects considered random. If an ANOVA indicated that significant ($P \leq 0.05$) differences existed among F_2–derived lines, transgressive segregation was tested with Student's t test ($\alpha = 0.05$) by comparing means of the most- and least-resistant F_2–derived lines to the means of each parent. Differences between parental means also were tested with the Student's t test ($\alpha = 0.05$). Broad-sense heritabilities (H_b^2) were estimated from expected mean squares (Fehr, 1987).

To identify lines with superior IDC resistance, a selection-threshold value was determined by first ranking the 145 F_2–derived lines on the basis of mean chlorosis scores at each location and combined over locations. The mean chlorosis score of the tenth-most resistant F_2–derived line was used as a selection-threshold value. Subsequently, F_2–derived lines with mean chlorosis scores equal to or less than the selection-threshold value were considered to possess superior IDC resistance.

Molecular Marker Evaluation

For each parent and F_2 line, leaf samples from 10 to 15 plants were harvested at the V4 stage of growth (Fehr and Caviness, 1981). Using a method adapted from Saghai-Maroof et al. (1984), DNA was extracted from macerated, freeze-dried leaf tissue with extraction buffer [1% (w/v) CTAB (cetyltrimethylammonium bromide), 50 m*M* Tris (2,3-Dibromo-1-propanol phosphate), 50 m*M* EDTA (ethylenediamine tetra-acetic acid), 0.7 NaCl, and 1 m*M* 1,10 phenanthroline]. The DNA was isolated from the extraction buffer with 24 parts chloroform and 1 part isoamyl alcohol solution and precipitated with 2/3 volume of isoproponal. The precipitated DNA was rinsed with 80% (v/v) ethanol and 15 m*M* ammonium acetate solution. The DNA was air-dried before resuspension in TE buffer (0.01 m*M* Tris, 0.01 m*M* EDTA) with 0.003% of RNase A.

One hundred and eight SSR markers (Table 1) genetically linked to eight previously identified QTLs (MLGs A1, B1, B2, G, H, I, L, and N) for IDC (Cregan et al., 1999; Lin et al., 1997, 2000a) were tested on P9254 and A97–770012 using procedures adapted from Cregan and Quigley (1997). For each marker, polymerase chain reactions (PCRs) were conducted in 20 μL-volumes of sterile, double-distilled water containing the following reagents: 0.2 m*M* of dATP (5′-adenylate triphosphate), dCTP (2′-deoxycytidine-5′-triphosphate), dGTP (2′-deoxyguanosine 5′-triphosphate), and dTTP (5′-thymidylate triphosphate); 1.5 m*M* $MgCl_2$; 20 m*M* Tris-HCl, 50 m*M* KCl; 0.5 U TAQ Polymerase; 45 ng genomic DNA; and 1.5 pmol of each SSR primer (forward and reverse). Forward and reverse primer sequences were obtained from SoyBase (http://www.soybase.org, verified 14 July 2005) for each SSR marker. Each reaction was subjected to touch-down PCR (Don et al., 1991) (PTC-200 DNA Engine, MJ Research, Inc.; Walthan, MA) using thermal-cycle conditions consisting of 94°C for 120 s followed by 12 cycles of 94°C for 60 s (denaturation), 52°C with a 1°C decrease for each successive cycle (range of 47 to 52°C) (annealing), and 68°C for 60 s (extension). Touch-down cycles were immediately followed by 35 cycles of 94°C for 30 s (denaturation), 47°C for 30 s (annealing), and 68°C for 30 s (extension), and followed with 68°C for 300 s. Amplification products were separated by electrophoresis on 2.5% (w/v) agarose gels incubated in buffer [40 m*M* Tris and 1 m*M* EDTA dissolved in 0.1% (v/v) glacial acetic acid] for 1.5 h at 180 V. Gels then were incubated in 0.0001% (w/v) ethidium bromide for 20 min before visualization of amplification products with ultraviolet light.

Markers with observable differences in the molecular weight of PCR products (i.e., alleles) between P9254 and A97–770012 were used to genotype the F_2 lines using the procedure described for parental genotypes. For each SSR marker, each individual F_2 line was classified into one of three genotypic classes: homozygous for P9254 allele (AA), heterozygous for both parental alleles (AB), or homozygous for A97–770012 allele (BB). For each marker, the occurrence of Mendelian segregation of alleles (1 AA:2 AB:1 BB) within the F_2 lines was tested using the chi-square test. Markers deviating from the expected allele segregation ratio ($P \leq 0.01$) were eliminated from the analysis.

Since all polymorphic markers identified in our study were associated with previously reported IDC QTLs, data from these markers were combined for analysis. Significant ($P \leq 0.05$) associations of genotypic classes with mean chlorosis score was determined with Type III (Partial) sums of squares in multiple-locus ANOVAs to identify SSR markers genetically linked with chlorosis expression. This approach gives the unique contribution for each marker after the correlated effects of the other markers are removed and this statistical approach reduces the rate of false positives relative to single-locus ANOVA (Snedecor and Cochran, 1989; Steel et al., 1997). Coefficients of determination (R^2) were reported for each marker significantly ($P \leq 0.05$) associated with chlorosis scores. For mean analysis, mean chlorosis score of selected F_2–derived lines was compared with the overall mean of the 145 F_2–derived lines using Dunnett's test ($\alpha = 0.05$).

Table 1. Simple sequence repeat (SSR) markers located in previously identified quantitative trait loci (QTL) for iron-deficiency chlorosis (IDC) resistance (Lin et al. 1997, 2000a).

Previously identified QTLs	SSR marker	Polymorphic
MLG† A1	Satt050	P‡
	Satt385	P
	Satt545	–
	Satt599	P
	Satt211	P*
	Satt511	–
	Satt236	–
	Satt225	–
	Satt200	–
	Satt174	–
MLG B1	Satt426	–
	Satt509	–
MLG B2	Satt304	–
	Satt083	–
	Satt601	–
	Satt318	–
	Sct_094	–
	Satt556	P
	Satt474	P
	Satt070	P
	Satt272	–
	Satt122	–
	Satt020	P
	Satt066	–
	Sct_064	–
	Satt534	P
	Satt063	–
	Satt560	–
MLG G	Satt505	–
	Satt138	–
	Satt400	–
	Satt199	–
	Satt012	P
	Satt503	–
	Satt517	–
	Satt288	P
	Satt472	–
	Satt191	–
	Sat_117	–
	Sct_187	–
MLG H	Satt541	–
	Satt469	–
	Sat_122	–
	Satt052	–
	Sat_118	–
	Satt314	–
	Satt279	–
	Satt222	–
	Satt253	–
	Satt302	–
	Satt142	–
	Satt293	P
	Satt181	P
	Satt317	P
	Satt434	–
MLG I	Satt239	–
	Satt496	–
	Satt354	–
	Sat_105	–
	Satt270	–
	Satt049	–
	Sat_104	P*
	Satt330	–
	Satt292	P
	Satt148	–
	Satt162	–
	Sct_189	–
	Satt440	–
MLG L	Satt143	–
	Sat_134	–
	Satt523	–
	Satt278	–
	Satt418	–
	Satt398	–
	Satt497	–
	Satt313	–
	Satt284	–
	Satt462	–
	Satt481	P*
	Satt156	–
	Sct_010	–
	Satt076	–
	Sat_113	–
	Satt561	–
	Satt527	–
	Satt166	–
	Satt448	P
	Sat_099	–
	Satt006	–
MLG N	Satt159	–
	Satt152	–
	Satt009	P
	Satt530	–
	Satt584	–
	Satt485	P
	Satt393	–
	Sat_084	–
	Satt125	–
	Sat_033	–
	Satt387	–
	Satt080	P
	Satt521	P
	Satt549	–
	Satt339	–
	Satt237	P
	Sat_091	P
	Satt257	–
	Satt410	–

(cont'd on next column)

Table 1. cont'd

*** Markers significantly associated with IDC resistance at $P \leq 0.05$.**
† MLG = molecular linkage group.
‡ P indicates those markers polymorphic between P9254 and A97–770012 on each MLG.

RESULTS

Phenotypic Evaluation for IDC Resistance

For parents and five check cultivars, there were significant ($P \leq 0.05$) differences among genotypes, and a significant interaction ($P \leq 0.05$) of location × year at individual locations and in the combined data (analysis not shown). At both locations, ranking of the five check cultivars for IDC resistance was similar to the previously established ranking (data not shown). This result indicated that foliar chlorosis expression resulted from iron deficiency.

In the overall analysis of chlorosis scores of the F_2–derived lines, there were no significant differences between locations and among years (Table 2). However, the location × year interaction was significant ($P \leq 0.01$), suggesting that environment is important in chlorosis expression. Within locations, years were significantly ($P \leq 0.05$) different, however, the absence of a line × year interaction suggested relative differences in chlorosis scores among F_2–derived lines were similar among years.

Although the parents did not differ from each other in chlorosis expression (Table 3), there were significant ($P \leq 0.01$) differences among F_2–derived lines within locations and in the overall analysis (Table 2). Transgressive segregation was also observed in the population (Table 2 and Fig. 1). Chlorosis score means of the F_2–derived lines ranged from 1.28 to 2.61 at Ames, 1.67 to 3.44 at Humboldt, and 1.64 to 2.89 for the overall data. At each location and across locations, mean chlorosis scores of the each parent were significantly ($\alpha = 0.05$)

Table 2. Analyses of variance of foliar chlorosis scores evaluated at the V4 stage in F_2–derived lines grown on calcareous soils in Ames and Humboldt during 2000, 2001, and 2002.

Source of variation†	df	Mean square‡
	Overall	
Year	2	22.31ns§
Location	1	76.54ns
Location × year	2	235.25**
Rep/(location × year)	12	11.20**
Line	144	1.22**
Line × year	288	0.49ns
Line × location	144	0.42ns
Line × location × year	288	0.42ns
Error	1727	0.41
	Ames	
Year	2	73.33*
Rep/year	6	12.06**
Line	144	0.79**
Line × year	288	0.43ns
Error	863	0.38
	Humboldt	
Year	2	184.26**
Rep/year	6	10.35**
Line	144	0.86**
Line × year	288	0.47ns
Error	863	0.44

* $P \leq 0.05$.
** $P \leq 0.01$.
† Year, location, and lines were considered random effects.
‡ Data combined across years and locations (overall) and within location (Ames or Humboldt).
§ ns = not significant.

Table 3. F_2–derived lines with superior iron-deficiency chlorosis resistance selected on the basis of mean chlorosis score during 2000, 2001, and 2002, with the combined data (overall). Marked in italic font are three lines (060, 095, and 130) that were selected consistently at each location and with overall means. Genotype for each line and parent is included for the Satt481 locus.

	Genotype†	Overall‡	Ames§	Humboldt§
F_2–derived lines				
Superior resistance				
017	BB	1.64	1.28	–
060	*BB*	*1.69*	*1.50*	*1.89*
095	*AA*	*1.69*	*1.50*	*1.89*
130	*BB*	*1.69*	*1.50*	*1.89*
146	BB	1.71	–	1.78
018	BB	1.72	–	1.83
066	BB	1.72	1.44	–
076	BB	1.72	1.33	–
092	BB	1.72	–	1.67
101	AA	1.72	1.44	–
122	AA	1.72	1.33	–
Selection-threshold value¶		1.72	1.50	1.89
Parents#				
P9254	AA	1.94	1.83	2.06
A97–770012	BB	2.08	2.06	2.11

† Genotype of each line at the Satt481 locus.
‡ Means combined over locations and years.
§ Means from three replications over 3 yr; means given only for lines also selected with the overall mean.
¶ Derived from the chlorosis score of the tenth-most resistant F_2–derived line.
# Mean chlorosis scores for the parents of F_2–derived lines.

different from the most-IDC susceptible line in the population at Ames, Humboldt, and overall (analysis not shown). In contrast, the parents were not different from the most-IDC resistant line at Humboldt or overall, except at Ames, where both parents differed ($\alpha = 0.05$) in mean chlorosis score with the most IDC-resistant line (analysis not shown).

Estimated H_b^2 for chlorosis scores was 63.6% for the overall data, similar to 62.3% at Ames and 62.1% at Humboldt. Because of significant differences among means of the F_2–derived lines according to ANOVA, selection of lines for IDC resistance and molecular marker analysis was done using the means of each location and overall data. Although there were no significant differences detected for location with the overall means, the individual location was still used to examine the consistency of performance across locations, which is an important consideration in actual breeding programs.

Selection-threshold values were 1.50 at Ames, 1.89 at Humboldt, and 1.72 for overall data (Table 3). Thirteen lines at Ames, 14 at Humboldt, and 11 lines overall expressed chlorosis scores equal to or less than the corresponding selection-thresholds. There were three lines (060, 095, and 130) identified as possessing superior IDC resistance at both locations and overall (Table 3). Only five lines at Ames and three at Humboldt were selected as having superior IDC resistance using the overall mean at each location.

Molecular Marker Evaluation

Of the 108 SSR markers genetically linked to previously identified QTLs for IDC resistance (Lin et al., 1997, 2000a), 24 markers (22.2%) were polymorphic between P9254 and A97–770012. All markers except one (Satt288) followed Mendelian segregation. Multiple-locus ANOVA indicated significant ($P \leq 0.05$) F values for Ames (1.48), Humboldt (1.59), and overall data (1.78). Of the 23 markers, only three were significantly ($P \leq 0.05$) associated with chlorosis scores using Ames, Humboldt, or overall data. The significant markers were Satt 211 (MLG A1), Satt481 (MLG L), and Sat_104 (MLG I) (Table 4). Significant ($P \leq 0.05$) R^2 values for the three markers ranged from 3.9 to 11.5%, with the greatest R^2 value for Satt481.

For Satt211, mean chlorosis scores for genotypic classes were significantly ($P \leq 0.05$) different only for Humboldt, where lines homozygous for P9254 allele (AA) showed the greatest IDC resistance (Table 4). For Satt481, the greatest IDC resistance was observed in lines homozygous for the A97–770012 allele (BB) at both locations and with the overall data. For Sat_104, the heterozygous (AB) genotype demonstrated the greatest IDC resistance in the overall mean. However, lines homozygous for the A97–770012 allele (BB) and heterozygous (AB) as Sat_104 demonstrated the same chlorosis scores at Humboldt. In a practical breeding situation, only the homozygotic state of Sat_104 would be useful in a self-pollinated and highly, genetically homozygous crop such as soybean.

The mean chlorosis scores of lines selected with individual markers were compared with the mean of the 145 F_2–derived lines within location and overall data (Table 5). Although selection of lines homozygous for the resistant allele at Satt211 and Sat_104 had no effect on population means, F_2–derived lines chosen with Satt 481 alone demonstrated a significantly lower ($\alpha = 0.05$) mean score of 0.11 to 0.14 units relative to the mean of the entire population of F_2–derived lines.

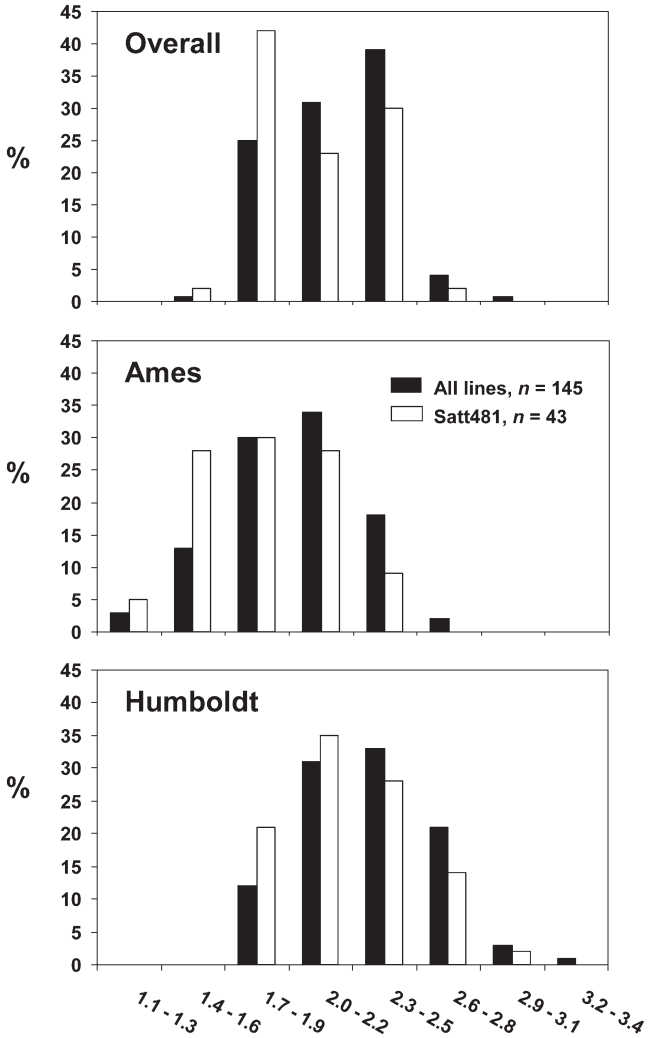

Fig. 1. Frequency distribution of chlorosis score means of F_2–derived lines. Distribution of means was compared between the entire breeding population, and lines were selected to possess resistance alleles at the Satt481 locus using data from Ames, Humboldt, and overall means.

In addition, when the distribution of chlorosis score means of F_2–derived lines was compared between the unselected population and the population of lines homozygous resistant for Satt481 (BB) (Fig. 1), there was a slight shift in the frequency of lines with superior resistance. For instance, for the overall means there was an 18-percentile increase in the number of lines, with chlorosis score means between 1.4 and 1.9 as a result of selection with Satt481. Similar results were observed at individual locations. Furthermore, at both locations, unselected and selected lines using Satt481 demonstrated normal distributions in the frequency of chlorosis score means.

DISCUSSION

Our objective was to determine whether SSR markers located in QTLs for IDC previously identified in other populations would be associated with resistance in a breeding population. We examined 108 SSR markers spanning eight QTLs for IDC resistance. Of the 24 markers for which parents of the breeding population were polymorphic, only three were associated with IDC resistance. Of the three, Satt481 was the only marker consistently associated with resistance in an environment-independent nature, despite the fact that Satt481 only explained about 12% of the total variation of IDC. When selections were done using Satt481, the mean of selected lines slightly improved relative to the unselected population. For this marker to be useful in breeding for IDC resistance, additional markers associated with resistance, combined with Satt481, may be required or phenotypic selection also would be needed.

Our study reflects the complex genetics of IDC resistance. Two genetic mechanisms for IDC resistance have been previously identified in soybean: a major gene with modifier genes and classical polygenic inheritance (Lin et al., 1997). In our population, the normal distribution in chlorosis scores observed in the F_2–derived lines suggested polygenic inheritance of IDC resistance, which may explain why major QTLs for resistance were not identified in the study.

Another likely explanation for identifying only one useful marker is that the desirable resistance alleles, espe-

Table 4. Mean chlorosis score of each genotypic class for simple sequence repeat markers Satt211, Satt481, and Sat_104 using Ames, Humboldt, and overall data from 145 F_2–derived lines.

		Genotypic classes†			
	MLG‡	AA	AB	BB	R^2
					%
Satt211	A1				
Overall§		2.11	2.18	2.19	2.2ns¶
Ames#		1.95	2.02	1.95	0.7ns
Humboldt#		2.26	2.33	2.40	4.2*
Satt481	L				
Overall		2.23	2.21	2.04	11.5**
Ames		2.05	2.04	1.85	8.6**
Humboldt		2.40	2.35	2.22	9.5**
Sat_104	I				
Overall		2.24	2.12	2.18	3.9*
Ames		2.05	1.93	2.04	2.8ns
Humboldt		2.41	2.30	2.30	4.2*

* $P \leq 0.05$.
** $P \leq 0.01$.
† Means of F_2–derived lines homozygous for P9254 allele (AA), heterozygous for each parent allele (AB), and homozygous for A97–770012 allele (BB).
‡ MLG = molecular linkage group.
§ Means based on data combined across locations and years.
¶ ns = not significant.
# Means based on combined data within location over years.

Table 5. Mean chlorosis scores at each location and overall data of lines selected using Satt211, Satt481, and Sat_104.

			Chlorosis scores†		
SSR marker	Genotype	n‡	Overall§	Ames¶	Humboldt¶
Satt211	AA	41	2.11ns#	1.95ns	2.26ns
Satt481	BB	43	2.04*	1.85*	2.22*
Sat_104	BB	30	2.18ns	2.04ns	2.31ns
F_2–derived lines††		145	2.16	1.99	2.33

† Means of F_2–derived lines homozygous for P9254 allele (AA) or homozygous for A97–770012 allele (BB). (*) within column indicates the mean is significantly ($\alpha = 0.05$) different from mean of the entire population of 145 F_2–derived lines according to Dunnett's Test.
‡ n = number of lines selected as possessing resistant genotype.
§ Means of selected F_2–derived lines combined across locations and years.
¶ Means of selected F_2–derived lines within location over years.
# ns = not significant.
†† Means of the entire population of lines across locations and at each location.

cially genes contributing major effects to phenotypic expression, could have become fixed in germplasm as breeders develop cultivars. Considering the parents used in our study represented elite lines with comparable IDC resistance, the possibility exists that the parents already shared favorable alleles for the major genes. Fixation of major genes may be supported by the lack of markers associated with resistance in MLG N, where a major gene for IDC resistance had been previously found, accounting for up to 80% of the total variation (Lin et al., 1997, 2000a). In our population, this major QTL was not detected, suggesting that we may be searching for modifier genes with minor effects on chlorosis expression, which would be difficult to detect. The low R^2–values observed for the three significant markers suggest that these markers mapped modifier genes with minor effects on resistance.

Although previous studies have demonstrated significant importance of the environment on genotype selection (Lin et al., 1997, 2000a), this was not statistically detected in our study. Still, environmental effects were evident in our population. Of the eleven-most resistant lines selected on the basis of chlorosis score means, only three lines were selected at both locations and with the overall mean. These observations suggest the role played by environment on chlorosis expression, which would also explain the intermediate heritability values for IDC observed in our study.

Despite the fact that Satt481 made a small contribution to the overall phenotypic variation in chlorosis score means, it is interesting to note that 73% of the top eleven-most resistant lines were homozygous resistant for Satt481. This allele was contributed by the A97–770012 parent, which had been developed specifically for IDC resistance. Lastly, although the selected lines were homozygous resistant at Satt481, a normal distribution in chlorosis score means was still observed for the lines, suggesting that several unidentified genes may be involved in IDC in the population.

Usefulness of Satt481 in a practical breeding situation will also be dependent on if Satt481 could be used in other breeding populations, and if additional markers could be identified. Work is underway to identify specific genes for IDC resistance using microarray experiments (O'Rourke and Shoemaker, 2005, personal communication). This genetic information could lead to gene-specific markers that may improve the effectiveness of MAS to increase IDC resistance in soybean.

ACKNOWLEDGMENTS

We would like to thank the Soybean Promotion Board for partial funding of this project. We also thank Ann Harris, Taylor Thompson, and Kelly Knavel for their assistance in preparing and genotyping the experimental lines, and Bruce Voss for his assistance during phenotypic evaluations. We also thank Nieves Rivera-Velez for her assistance in growing the population in Puerto Rico at the Iowa State University Soybean Breeding Research site.

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